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Reduction of Abscisic Acid Content and Induction of Sprouting in Potato, *Solanum tuberosum* L., by Thidiazuron

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Abstract. The effect of [(*N*-phenyl-*N'*-1,2,3-thidiazol-5-ylurea)] (thidiazuron) on sprouting of potato (*Solanum tuberosum* L.) tubers and the role of ABA in bud break and subsequent bud growth were studied. Abscisic acid (ABA) was quantitated by enzyme-linked immunosorbent assay (ELISA) from the peel of potato tubers. The ELISA results were also validated by gas chromatography–electron capture detector and confirmed by gas chromatography–mass spectrometry and by a lettuce hypocotyl bioassay. The degree of rest in the tubers was associated with ABA content in the peel. Basal portion (where tuber was attached to mother plant) contained the highest amount of ABA. Thidiazuron reduced ABA content and induced potato tuber sprouting. Exogenously applied ABA stimulated growth of buds that had emerged from dormancy.

Deep dormancy of potato tubers is related to the presence of the "inhibitor-β" complex (Hemberg 1967). Levels of inhibitors in peel were correlated with the state of dormancy of the buds (Hemberg 1967). The principal inhibitor in potato tubers is abscisic acid (ABA) (Letham 1978). Incubation of tubers at low temperatures (2°C) decreases inhibitor levels and increases the rate of sprouting and the growth of the sprouts (Thomas and Wurr 1976). Auxin (Rappaport et al. 1965), gibberellins (Boo 1961, Hawthorne 1969), and cytokinins (Hemberg 1970, Tsukamoto 1972) also reduce inhibitor levels markedly, increase endogenous promotor levels, and induce dormancy break of potato tubers. Obhlidalova et al. (1980) also demonstrated the increases in both gibberellin and cytokinin levels during dormancy break of potato tubers. A move-

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ment of gibberellin activity from base to apex was also found to be associated with sprouting.

The plant bioregulator [(*N*-phenyl-*N'*-1,2,3-thidiazol-5-ylurea)] (thidiazuron; Dropp; SN49537; TDZ) has the capacity to release apple lateral buds from dormancy and induce metabolic change (Wang et al. 1986, 1987). Thidiazuron is a urea derivative that stimulates the conversion of cytokinin nucleotides to nucleosides. It has been shown to exhibit cytokininlike activity in bioassay systems and to promote growth in cytokinin-dependent callus culture in *Phaseolus*, soybean, and tobacco (Capelle et al. 1983, Mok et al. 1982, Thomas and Katterman 1986). The present study was undertaken to investigate the effect of thidiazuron on ABA levels and sprouting of potato tubers and also to determine the role of ABA in bud break and subsequent bud growth.

Materials and Methods

Plant Materials and Treatments

Potato (*Solanum tuberosum* L.) tubers of cv. Kennebec were planted on April 30, 1986 and 1987, at Beltsville, MD. The field was broadcast with 500 lb of fertilizer (5-10-10) per acre before planting and side-dressed again with the same fertilizer (500 lb/acre) after planting. Potatoes were harvested on early August and used in the experiments without storage. About 1.5 cm of basal portion (attached to mother plant) of a group of potato tubers was excised. All the tubers were equally divided longitudinally. Thidiazuron and ABA were prepared in 2.5% DMSO plus 0.5% Tween-20, and thidiazuron was applied directly to the eyes with a brush until runoff. Tubers were planted in 8"-pots containing potting soil and placed in the greenhouse. In one group of tubers, 3 weeks after thidiazuron treatment, the emerged buds were then treated with various concentrations of ABA. The buds sprouting from the upper portion of tubers were defined upper-portion buds, whereas the buds sprouting from the lower portion of tubers were defined lower-portion buds. Radiation sources in the greenhouse consisted of natural daylight. Temperatures were approximately 25°C during the day and 20°C at night.

Extraction and Purification of ABA

All manipulations were carried out under dim green light in a cold room (4°C). Potato tuber peel tissue was extracted with 80% methanol [containing 10 mg/L of 2,6-di-*t*-butyl-4-methyl phenol (BHT)] (pH 4.8) for 72 h at 4°C with intermittent stirrings, and about 15,000 dpm of [^{3}H]ABA (22.5 Ci/mmol; Amersham, Arlington Heights, IL) was added to each extract to determine losses during the purification procedure. The extract solution was replaced with a fresh solvent ever 24 h. The combined extract was evaporated under vacuum (40°C) to an aqueous solution. The aqueous solution was then centrifuged at 3000*g* for 15 min at 0°C. An equal volume of 0.1 M phosphate buffer (pH 8.0) was added to the supernatant. The pH of the solution was adjusted to 8.0 with 1 N NaOH.

The solution was then partitioned three times with an equal volume of petroleum ether. The pH of the aqueous solution was then acidified to pH 2.8 with 6 N HCl, and the solution was extracted with ethyl acetate three times. Hexane was added to the ethyl acetate fraction to make a hexane/ethyl acetate ratio of 7:3. After centrifugation at 200*g* for 5 min, the lower water layer was discarded. The supernatant was passed through a Baker 10 SPE 3-ml column prepacked with 500 mg of silica gel (Powell and Maybee 1985). ABA was eluted with 2 ml methanol/acetonitrile (1:3) and evaporated to dryness under N_2. Overall recovery of [^{3}H]ABA was between 60% and 70%. The ABA residue was taken up with phosphate-buffered saline for enzyme-linked immunosorbent assay (ELISA) quantitation (Phytodeteck 1985, Weiler 1982) and lettuce hypocotyl bioassay (Bakken and Boe 1982) and was methylated with diazomethane (Fales et al. 1973) for gas chromatography–selected ion monitoring (GC-SIM) (Wang et al. 1987) and gas chromatography–electron capture (GC-EC) analysis (Rivier et al. 1977, Leroux et al. 1985). All experiments were repeated three times with three replications for each treatment.

Enzyme-Linked Immunosorbent Assay (ELISA)

Monoclonal antibody (IgG_1; Idetek, San Bruno CA) was used to detect and quantitate endogenous free (+) ABA in the potato tuber peel extract (Phytodetek 1985, Weiler 1982). This antibody is specific only for free *cis,trans*(+)ABA and has no cross-reactivity of antibody with *cis,trans*(−)-ABA, *trans,trans*(+)ABA, *cis,trans*(+)ABA-D-glucopyranosyl ester, *cis,trans*(+)ABA-*cis-diol*, xanthoxin, and all-*trans*-farnesol, and very light (<0.1%) cross-reactivity with *cis,trans*(+)ABA-Me, phaseic acid, dihydrophaseic acid. The lyophilized monoclonal antibody was dissolved in TBS (25 mM) TRIS-HCl, 100 mM NaCl, 1 mM $MgCl_2$, 0.1% sodium azide, pH 7.5). The alkaline phosphatase-labeled ABA (1.25 μg/ml; Idetek) was prepared according to instructions and stored in 25 mM TRIS-HCl, 1 mM $MgCl_2$, 0.1% sodium azide, 0.1% gelatin, pH 7.5, at 4°C (Phytodetek 1985). Each well in the 96-well polystyrene plate was coated with 200 μl of a 5 μg/ml monospecific rabbit anti–mouse IgG_1 solution in 50 mM $NaHCO_3$, pH 9.6, for 16 h at 4°C. After decanting and thorough rinsing of the wells with water, individual wells in the plate were filled with 100 μl of diluted silica column purified samples or with *cis,trans*(+)ABA (Sigma) standard solutions (0.0–2.0 pmole along with 100 pmole of ABA for measurement of nonspecific binding).

This was followed by 100 μl of diluted enzyme-labeled ABA (alkaline phosphate) and incubated for 3 h at 4°C. The plates were then decanted and rinsed with water (3 times). The activity of the enzyme conjugate bound to the polystyrene-absorbed antibodies was then determined using 0.2 ml of *p*-nitrophenyl phosphate (1 mg/ml in 50 mM $NaHCO_3$, pH 9.6) as substrate. The enzyme reaction was allowed to proceed at 37°C for 1 h and was stopped with 50 μl of 1 M KOH per well. After 5 min, the readings were taken with a vertical light path photometer at 405 nm. Sample location on the ELISA plate was randomized to minimize edge effect. Each sample was analyzed at two or more dilutions. Calculations were done as described (Phytodetek 1985, Weiler 1982). The B/B°

reading corresponded only to the log-linear part of the standard curve. The data calculated from the log-linear portion of the standard curve were closer to the values determined by GC-EC than values calculated from dilutions falling on curvilinear portions of the standard curve.

Lettuce Hypocotyl Bioassay

The solutions of (+) ABA standards (range 0.001–100 μg/ml) and the silica column-purified extracts of potato peel (dissolved in methanol) were separately streaked on Whatman No. 1 paper and chromatographed with a solvent of 1-butanol:ethanol:water:ammonia (58:18:14:5). The chromatogram was then assayed using the lettuce (*Lactuca sativa* cv. Black Seeded Simpson) hypocotyl elongation test (Bakken and Boe 1982).

Statistical Analysis

Data were analyzed statistically as a randomized complete block experiment having a repeated measurement design using analysis of variance.

Results and Discussion

Thidiazuron stimulated bud break at all concentrations. The optimum dosage was 50 μM for inducing sprouting and subsequent growth of sprouts (Figs. 1, 2). This indicates that thidiazuron could induce sprouting in potato tubers. The time course for tubers to respond to various dosage of thidiazuron is shown in Fig. 2. The effects of attachment of the basal portion of potato on bud break and bud growth are presented in Table 1. The data showed that excision of the basal portion of the tubers increased the percentage of sprouting, the number of sprouted buds per eye, and fresh weight per bud. This was probably due to high amount of ABA in the basal portion of tubers (Table 2). The data indicate that dormancy is related to endogenous inhibitor content, as suggested by Thomas (1981). ABA content in upper and lower portions of tubers decreased after the basal portion was excised (Table 2). Removal of the basal part of the tuber apparently eliminated the source for upward transport of the inhibitor.

Thidiazuron treatment caused a reduction in ABA levels (Table 2) and increased the percentage of sprouting, number of sprouted buds per eye, and fresh weight per bud (Table 1). Possible involvement of inhibitor-promotor interaction in the regulation of potato tuber dormancy has been reported (Thomas and Wurr 1976). With the development of buds, there was a concomitant increase in cytokinin activity (Thomas 1981). The induction of potato sprouting with thidiazuron may be also due to an increase in the ratio of promotor (cytokinin)/inhibitor (ABA) in the tubers.

The amount of ABA in basal portion of tubers was 3.97 and 3.67 nmol/g fresh weight as determined by ELISA and GC-EC, respectively. The selected ion monitoring (SIM) responses at m/z 190 for the methyl ester of ABA in the

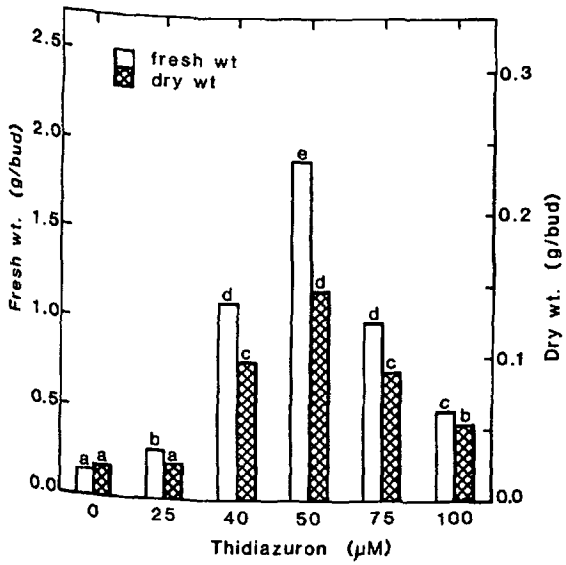

Fig. 1. Effect of various concentrations of thidiazuron on potato sprout weights. Mean separation by Duncan's multiple-range test, 5% level. Measurements were taken 4 weeks after treatment. Within each measurement parameter (fresh weight/bud or dry weight/bud), points followed by the same letter do not differ significantly.

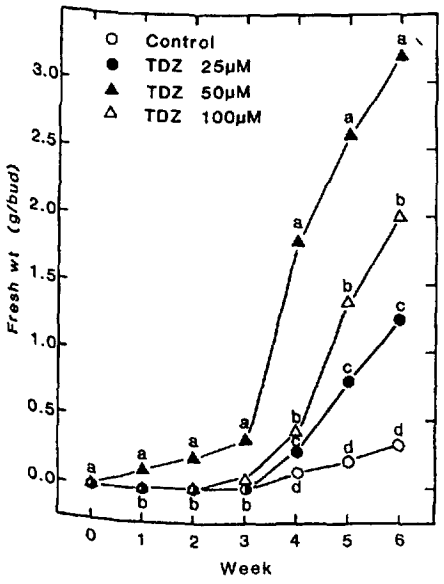

Fig. 2. Time course of the effect of various concentrations of thidiazuron (TDZ) on potato sprouting. Mean separation by Duncan's multiple-range test, 5% level. Within each sampling time, points followed by the same letter do not differ significantly.

extract of potato tubers and authentic ABA were identical (data not shown). The fraction (R_f = 0.8) from the purified tissue extract separated by paper chromatography that showed the most inhibitory activity in lettuce hypocotyl bioassay coincided with that of synthetic ABA (data not shown). From the above information, our results indicated that the inhibitory compound from potato tuber peel was ABA and ABA levels to be correlated to inhibition of

Table 1. Effect of attachment of the basal portion of potatoes on thidiazuron-induced bud break and bud growth on the tubers.

Treatment (basal portion)	Tuber portion	Control			Thidiazuron-treated		
		% sprouting	Number buds/eye	FW mg/bud	% sprouting	Number bud/eye	FW mg/bud
Without	Upper	15.3a	2.00a	47.9b	75.0a	2.75a	280.0c
With	Upper	5.2b	1.00b	36.3c	62.5b	2.25b	227.0d
Without	Lower	14.7a	1.00b	129.3a	81.5a	1.75c	612.9a
With	Lower	0.0c	0.00c	0.0d	37.5c	1.00d	550.0b

Thidiazuron (50 μM) was applied to the buds before tubers were planted in the soil. Measurements were taken 3 weeks after treatment. Means in column separated by Duncan's multiple-range test, 5% level.

Table 2. ABA content in potato peel from different portions of the tuber with or without thidiazuron treatment and with or without removal of basal portion.

Portion of tuber	ABA (nmol/g fresh weight)			
	Control		Thidiazuron-treated	
	Intact	Without basal	Intact	Without basal
Basal portion	3.97g	—	2.98f	—
Lower half	2.45e	1.80bc	2.06d	1.01a
Upper half	2.00cd	1.73b	1.58b	0.89a

Thidiazuron (50 μM) treatment was applied at the beginning of the experiment. Peels were collected from potato tubers 3 weeks after treatment. ABA in peel was determined by ELISA. Mean separation by Duncan's multiple-range test, 5% level.

sprouting. Exogenously applied ABA (25 and 50 μM) enhanced growth of sprouts that had emerged from dormancy, and a higher ABA (100 μM) concentration was inhibitory (Fig. 3). It appears that ABA inhibits bud break but advances sprout growth. Stimulation of growth in decapitated bean plants and lateral buds of apple by ABA has also been reported (Hartung and Steigerwald 1977, Wang et al. 1987).

Conclusion

The data presented here indicate that the basal portion of the potato tubers is the main source of inhibitors and that it inhibits the sprouting of buds throughout the rest of the tuber. The inhibitor was identified as ABA by ELISA, GC-EC, and lettuce hypocotyl elongation bioassay. Thidiazuron reduced ABA content and induced sprouting of potato tubers in deep rest. Since thidiazuron has also been shown to exhibit cytokininlike activity, it is apparent that dormancy is regulated not only by growth inhibitors but also by promotors that enhance the resumption of growth. Exogenously applied ABA stimulated

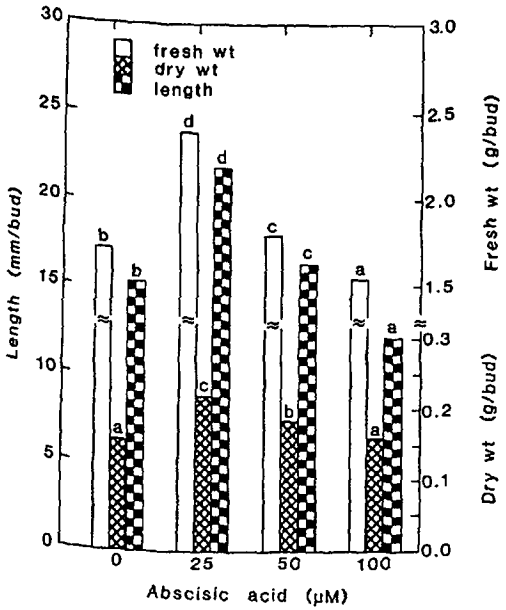

Fig. 3. Effect of ABA on the growth of thidiazuron-induced potato sprouts. Potato eyes from intact tuber were treated with 50 μM thidiazuron. Sprouting was evident after 3 weeks. Three sprouted buds with comparable size on each of five tubers were used for treatment with each ABA concentration. Bud length, fresh weight, and dry weight were measured 8 days after ABA treatment. Mean separation by Duncan's multiple-range test, 5% level. Within each measurement parameter (bud length, fresh weight/bud, or dry weight/bud), points followed by the same letter do not differ significantly.

growth of buds that had emerged from dormancy. Further study is needed to investigate the mechanism of the effect of exogenous ABA on bud growth.

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